CLAIMS

What is claimed is:

1. An assay for screening a test substance for HIF inhibitory activity of an ouabain-resistant Na^+-K^+-ATPase, comprising the steps of:

(a) contacting liposomes containing, or cells expressing or containing, the ouabain-resistant Na^+-K^+-ATPase with the test substance in the presence of a compound comprising isotopic Rb^+, under suitable conditions for isotopic Rb^+ uptake by the liposomes or the cells;

(b) measuring or detecting the amount of the isotopic Rb^+ present in the liposomes or the cells; and

(c) comparing the isotopic Rb^+ present in the liposomes or cells measured or detected with measured or detected isotopic Rb^+ obtained by contacting liposomes containing, or cells containing or expressing, ouabain-resistant Na^+-K^+-ATPase with HIF in the presence of a compound comprising isotopic Rb^+, under comparable conditions, thereby determining whether the test substance exhibits HIF inhibitory activity.

2. The method of Claim 1, further comprising the step of contacting ouabain-resistant Na^+-K^+-ATPase with HIF under suitable conditions in an aqueous medium for ouabain-resistant Na^+-K^+-ATPase activity, wherein said activity is measured or detected, thereby providing the measurement or detection to be compared with the test substance.

3. The method of Claim 1, wherein the ouabain-resistant Na^+-K^+-ATPase is obtained by substituting one or both border amino acid residues of the H1-H2 extracellular domain of α subunit of an ouabain-sensitive Na^+-K^+-ATPase with a charged amino acid residue.

4. The method of Claim 1, wherein the isotopic Rb^+ is $^{86}Rb^+$.

5. The method of Claim 1, wherein the isotopic Rb^+ present in the liposomes containing the ouabain-resistant Na^+-K^+-ATPase is separated from the unincorporated or liberated compound comprising isotopic Rb^+ by chromatography.

6. The method of Claim 1, wherein the isotopic Rb^+ present in the cells containing the ouabain-resistant Na^+-K^+-ATPase is separated from the unincorporated or liberated compound comprising isotopic Rb^+ by centrifugation through an oil layer.

7. The method of Claim 6, wherein the oil layer is a phthalate oil layer.

8. The method of Claim 4, wherein the amount of $^{86}Rb^+$ present in the liposomes or the cells containing the ouabain-resistant Na^+-K^+-ATPase is measured by a gamma counter.

9. The method of Claim 1, wherein the ouabain-resistant Na^+-K^+-ATPase is isolated from a target cell.

10. The method of Claim 9, wherein the target cell is selected from the group consisting of a kidney cell, a heart cell, a pineal gland cell, a skeletal muscle cell, a retina horizontal cell, a retina Muller cell, a brain cortical astrocyte, a cerebellar granule neuron, a cortical neuron and a Hippocampal neuron.

11. The method of Claim 9, wherein the target cell is derived from a patient exhibiting a disease state that is related to dysfunction of the ouabain-resistant

Na^+-K^+-ATPase in said target cell, thereby determining whether said target cell will respond to the test substance.

12. The method of Claim 11, wherein the disease state is selected from the group consisting of a cardiac malfunction in which a treatment to produce a positive inotropic effect is desired or needed, an edematous disorder and hypotension.

13. The method of Claim 12, wherein the cardiac malfunction is selected from the group consisting of congestive heart failure, paroxysmal atrial tachycardia and atrial fibrillation.

14. The method of Claim 12, wherein the edematous disorder is selected from the group consisting of congestive heart failure, cirrhosis of the liver and nephrotic syndrome.

15. The method of Claim 1, wherein the ouabain-resistant Na^+-K^+-ATPase is selected from the group consisting of a rodent, toad and butterfly α1 Na^+-K^+-ATPase.

16. The method of Claim 15, wherein the ouabain-resistant Na^+-K^+-ATPase is a rat kidney α1 ouabain-resistant Na^+-K^+-ATPase.

17. The method of Claim 15, wherein the ouabain-resistant Na^+-K^+-ATPase is a *Bufo marinus* α1 ouabain-resistant Na^+-K^+-ATPase.

18. The method of Claim 15, wherein the ouabain-resistant Na^+-K^+-ATPase is a *Danaus plexippus* α1 Na^+-K^+-ATPase.

19. The method of Claim 1, wherein the HIF is isolated from bovine hypothalamus.

20. A method for screening a test substance other than ouabain, for HIF-like inhibitory activity of an ouabain-resistant Na^+-K^+-ATPase, comprising the steps of:

(a) contacting the ouabain-resistant Na^+-K^+-ATPase with the test substance under suitable conditions for measuring or detecting ouabain-resistant Na^+-K^+-ATPase activity;

(b) measuring or detecting ATPase activity of the test substance; and

(c) comparing the activity of the ouabain-resistant Na^+-K^+-ATPase measured or detected in step (b) with the ouabain-resistant Na^+-K^+-ATPase activity of HIF under comparable conditions, thereby determining whether the test substance exhibits HIF-like inhibitory activity,

wherein the ouabain-resistant Na^+-K^+-ATPase is obtained by substituting one or both border amino acid residues of the H1-H2 extracellular domain of α subunit of an ouabain-resistant Na^+-K^+-ATPase with a charged amino acid residue.

21. A method for screening a test substance other than ouabain, for HIF-like inhibitory activity of an ouabain-resistant Na^+-K^+-ATPase, comprising the steps of:

(a) contacting the ouabain-resistant Na^+-K^+-ATPase with the test substance under suitable conditions for measuring or detecting ouabain-resistant Na^+-K^+-ATPase activity;

(b) measuring or detecting ATPase activity of the test substance; and

(c) comparing the activity of the ouabain-resistant Na^+-K^+-ATPase measured or detected in step (b) with the ouabain-resistant Na^+-K^+-ATPase activity of HIF under comparable conditions, thereby determining whether the test substance exhibits HIF-like inhibitory activity,

wherein the target cell is derived from a patient exhibiting a disease state that is related to dysfunction of the ouabain-resistant Na^+-K^+-ATPase in said target cell, thereby determining whether said target cell will respond to the test substance.

22. The method of Claim 21, wherein the disease state is selected from the group consisting of a cardiac malfunction in which a treatment to produce a positive inotropic effect is desired or needed, an edematous disorder and hypotension.

23. The method of Claim 22, wherein the cardiac malfunction is selected from the group consisting of congestive heart failure, paroxysmal atrial tachycardia and atrial fibrillation.

24. The method of Claim 22, wherein the edematous disorder is selected from the group consisting of congestive heart failure, cirrhosis of the liver and nephrotic syndrome.

25. A method for screening a test substance for HIF-like inhibitory activity of an ouabain-resistant Na^+-K^+-ATPase, comprising the steps of:

(a) contacting the ouabain-resistant Na^+-K^+-ATPase with the test substance in the presence of ATP, wherein the terminal phosphate (P_3) is labeled, under suitable conditions for measuring or detecting liberated labeled P_3;

(b) measuring or detecting liberated labeled P_3; and

(c) comparing the measured or detected liberated labeled P_3 with the measured or detected labeled P_3 that is liberated by contacting ouabain-resistant Na^+-K^+-ATPase with HIF in the presence of ATP, wherein the terminal phosphate is labeled, under comparable conditions, thereby determining whether the test substance exhibits HIF-like inhibitory activity,

wherein the ouabain-resistant Na^+-K^+-ATPase is obtained by substituting one or both border amino acid residues of the H1-H2 extracellular domain of α subunit of an ouabain-resistant Na^+-K^+-ATPase with a charged amino acid residue.

26. A method for screening a test substance for HIF-like inhibitory activity of an ouabain-resistant Na^+-K^+-ATPase, comprising the steps of:

(a) contacting the ouabain-resistant Na^+-K^+-ATPase with the test substance in the presence of ATP, wherein the terminal phosphate (P_3) is labeled, under suitable conditions for measuring or detecting liberated labeled P_3;

(b) measuring or detecting liberated labeled P_3; and

(c) comparing the measured or detected liberated labeled P_3 with the measured or detected labeled P_3 that is liberated by contacting ouabain-resistant Na^+-K^+-ATPase with HIF in the presence of ATP, wherein the terminal phosphate is labeled, under comparable conditions, thereby determining whether the test substance exhibits HIF-like inhibitory activity,

wherein the target cell is derived from a patient exhibiting a disease state that is related to dysfunction of the ouabain-resistant Na^+-K^+-ATPase in said target cell, thereby determining whether said target cell will respond to the test substance.

27. The method of Claim 26, wherein the disease state is selected from the group consisting of a cardiac malfunction in which a treatment to produce a positive inotropic effect is desired or needed, an edematous disorder and hypotension.

28. The method of Claim 27, wherein the cardiac malfunction is selected from the group consisting of congestive heart failure, paroxysmal atrial tachycardia and atrial fibrillation.

29. The method of Claim 27, wherein the edematous disorder is selected from the group consisting of congestive heart failure, cirrhosis of the liver and nephrotic syndrome.

30. A kit, comprising:

(a) an isolated ouabain-resistant Na^+-K^+-ATPase, or reconsituted liposomes or cells containing an ouabain-resistant Na^+-K^+-ATPase;

(b) HIF;

(c) a non-ATP substrate of said isolated ouabain-resistant Na^+-K^+-ATPase;

(d) ATP or labeled ATP; and

(e) a compound containing isotopic Rb^+.

31. A kit comprising:

(a) an isolated ouabain-resistant Na^+-K^+-ATPase;

(b) ATP in which the P_3 is labeled; and

(c) HIF.

32. A kit, comprising:

(a) reconstituted liposomes containing, or cells containing or expressing, an ouabain-resistant Na^+-K^+-ATPase;

(b) a compound containing isotopic Rb^+; and

(c) HIF.

33. A biologically active substance identified by the method of Claim 1.

34. A method of treating or preventing a disease or disorder which comprises administering, to a subject to which such treatment or prevention is needed, an effective amount of a biologically active substance identified by the assay of Claim 1, as exhibiting HIF inhibitory activity.

35. The method of Claim 34, wherein the disease or disorder is selected from the group consisting of a cardiac malfunction in which a treatment to produce a

positive inotropic effect is desired or needed, an edematous disorder and hypotension.

36. A method of treating or preventing a disease or disorder which comprises administering, to a subject to which such treatment or prevention is needed, an effective amount of a biologically active substance identified by a method comprising the steps of:

(a) contacting the ouabain-resistant Na^+-K^+-ATPase with the test substance under suitable conditions for measuring or detecting ouabain-resistant Na^+-K^+-ATPase activity;

(b) measuring or detecting ATPase activity of the test substance; and

(c) comparing the activity of the ouabain-resistant Na^+-K^+-ATPase measured or detected in step (b) with the ouabain-resistant Na^+-K^+-ATPase activity of HIF under comparable conditions, thereby determining whether the test substance exhibits HIF-like inhibitory activity.

37. A method of treating or preventing a disease or disorder which comprises administering, to a subject to which such treatment or prevention is needed, an effective amount of a biologically active substance identified by a method comprising the steps of:

(a) contacting the ouabain-resistant Na^+-K^+-ATPase with the test substance in the presence of ATP, wherein the terminal phosphate (P_3) is labeled, under suitable conditions for measuring or detecting liberated labeled P_3;

(b) measuring or detecting liberated labeled P_3; and

(c) comparing the measured or detected liberated labeled P_3 with the measured or detected labeled P_3 that is liberated by contacting ouabain-resistant Na^+-K^+-ATPase with HIF in the presence of ATP, wherein the terminal phosphate is labeled, under comparable conditions, thereby determining whether the test substance exhibits HIF-like inhibitory activity.